

# Histidylation by yeast HisRS of tRNA or tRNA-like structure relies on residues – 1 and 73 but is dependent on the RNA context

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## ABSTRACT

**Residue G<sub>-1</sub> and discriminator base C<sub>73</sub> are the major histidine identity elements in prokaryotes. Here we evaluate the importance of these two nucleotides in yeast histidine aminoacylation identity. Deletion of G<sub>-1</sub> in yeast tRNA<sup>His</sup> transcript leads to a drastic loss of histidylation specificity (about 500-fold). Mutation of discriminator base A<sub>73</sub>, common to all yeast tRNA<sup>His</sup> species, into G<sub>73</sub> has a more moderate but still significant effect with a 22-fold decrease in histidylation specificity. Changes at position 36 in the anticodon loop has negligible effect on histidylation. The role of residues – 1 and 73 for specific aminoacylation by yeast HisRS was further investigated by studying the histidylation capacities of seven minihelices derived from the Turnip Yellow Mosaic Virus tRNA-like structure. Changes in the nature of nucleotides – 1 and 73 modulate this activity but do not suppress it. The optimal mini-substrate for HisRS presents a G·A mismatch at the position equivalent to residues G<sub>-1</sub>·A<sub>73</sub> in yeast tRNA<sup>His</sup>, confirms the importance of this structural feature in yeast histidine identity. The fact that the minisubstrates contain a pseudoknot in which position – 1 is mimicked by an internal nucleotide from the pseudoknot highlights further the necessity of a stacking interaction of this position over the amino acid accepting branch of the tRNA during the aminoacylation process. Individual transplantation of G<sub>-1</sub> or A<sub>73</sub> into yeast tRNA<sup>Asp</sup> transcript improves the histidylation efficiency of the engineered tRNA<sup>Asp</sup>. However, a tRNA<sup>Asp</sup> transcript presenting simultaneously both residues G<sub>-1</sub> and A<sub>73</sub> becomes a less good substrate for HisRS, suggesting the importance of the structural context and/or the presence of anti-determinants for an optimal expression of these two identity elements.**

## INTRODUCTION

It is at present a well accepted view that specific aminoacylation of transfer ribonucleic acids (tRNAs) is mediated by a small set of identity nucleotides that most often interact with the cognate aminoacyl-tRNA synthetase (aaRS) and, by negative signals that prevent the tRNAs to be aminoacylated by non-cognate synthetases. Major identity nucleotides are known for all *Escherichia coli* tRNAs and several tRNAs in the eukaryotic kingdom [reviewed in e.g. (1–3)]. Much less is known about negative signals that have only been described explicitly in a few systems. In some cases, they have been correlated with the presence of modified nucleotides (4, 5). Expression of these identity elements are often modulated by structural elements present within the tRNA architecture (6–10). Identity nucleotides are searched *in vitro* by comparison of the aminoacylation capacities of wild-type and mutated tRNAs. Explicit proof of the contribution of nucleotides to identity comes from transplantation experiments in which the putative elements are inserted in a new tRNA framework with the expectation that the chimeric molecule acquires the new specificity. Optimal catalytic efficiency is obtained if no conformational or sequence context effects interfere with the expression of the specificity in the transplanted molecule.

Residues required for specifying histidylation in tRNAs have already been investigated in *E. coli* by both *in vitro* and *in vivo* approaches. Residue G<sub>-1</sub>, only present in tRNA<sup>His</sup> species, and the discriminator base C<sub>73</sub> specific for prokaryotic histidine tRNAs, have been found crucial for this identity (11, 12). The importance of these two residues, which likely are paired, was confirmed by the capacity of a microhelix derived from the amino acid acceptor arm of *E. coli* tRNA<sup>His</sup> containing base-pair G<sub>-1</sub>·C<sub>73</sub> to be histidylated, indicating also that the anticodon branch is not essential for *E. coli* histidyl-tRNA synthetase (HisRS) (13). Introduction of G<sub>-1</sub> and C<sub>73</sub> into a microhelix derived from tRNA<sup>Ala</sup> allowed its efficient histidylation, confirming the importance of this unique base-pair in histidylation identity. However, further transplantations of this element into

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other microhelices showed that its expression is modulated by the sequence context of the microhelix, and particularly by the 2·71 and 3·70 base-pairs which are minor histidine determinants (14).

Although all tRNA<sup>His</sup> molecules sequenced to date present a residue G<sub>-1</sub> (with one exception) (15), the explicit involvement of this residue in histidine identity has not been proven in organisms other than *E.coli*. Moreover, the nature of the discriminator base in tRNA<sup>His</sup> is different in prokaryotes and eukaryotes, so that it can be questioned about the uniqueness of histidine identity rules in living kingdoms. In this paper, we consider the case of yeast HisRS. Besides its cognate tRNA, this enzyme charges also viral tRNA-like structures (16–20) as well as a pseudoknot-containing minihelix recapitulating the amino acid acceptor branch of the Turnip Yellow Mosaic Virus (TYMV) tRNA-like structure (18).

Here we describe the histidylating capacities of a series of RNA transcripts derived from yeast tRNA<sup>His</sup>, yeast tRNA<sup>Asp</sup> and TYMV RNA. Our data indicate that in both tRNA and tRNA-like structural frameworks, the coupling between discriminator nucleotide N<sub>73</sub> and residue G<sub>-1</sub>, or its mimick, governs histidine identity toward the yeast enzyme.

## MATERIALS AND METHODS

### Materials

Yeast HisRS was an enzyme preparation enriched by chromatographies on DEAE-cellulose, hydroxyapatite and phosphocellulose (21). T7 RNA polymerase was prepared from an over-producing strain harbouring plasmide pBl21 according to (22). L-[<sup>14</sup>C] histidine (300 mCi/mmol) was from Amersham France (Les Ulis) and adjusted to a specific activity of 400 cpm/pmol with unlabelled aminoacid. Restriction enzymes *Bst*NI and *Eco*T22I were purchased from New England Biolabs (Beverly, MA, USA) and United States Biochemical Corporation (Cleveland, OH, USA) respectively. RNasin was from Promega (Madison, WI, USA).

### Cloning and *in vitro* transcription of synthetic yeast tRNA<sup>His</sup> genes

Ten synthetic overlapping deoxyoligonucleotides, corresponding to the consensus sequence of the T7 RNA polymerase promoter and to the sequence of yeast tRNA<sup>His</sup><sub>1</sub> (23) were hybridized and inserted into pUC119 linearised with *Bam*HI and *Hind*III as described for yeast tRNA<sup>Asp</sup> synthetic gene (24). An *Eco*T22I restriction site was introduced instead of *Bst*NI since there is an internal *Bst*NI restriction site in the tRNA sequence. Three variants of the tRNA<sup>His</sup> gene were cloned by replacing two overlapping oligonucleotides with their corresponding mutated versions. Transcriptions of these genes yield tRNA<sup>His</sup> transcripts with either a deletion of residue G at position -1, a mutation at position 36 (G into C) or a mutation at position 73 (A into G). Transcriptions of tRNA<sup>His</sup> genes (wild-type and mutated) were performed by incubation of *Eco*T22I-linearised plasmid (8 µg) with 450 units of T7 RNA polymerase in 40 mM Tris-HCl pH 8.0, 22 mM MgCl<sub>2</sub>, 1 mM spermidine, 5 mM dithioerythritol, 0.01% Triton X100, 120 units RNasin, 4 mM of each nucleoside triphosphate and 16 mM GMP in 150 µl for 3 hours at 37°C. After phenol extraction, transcripts were ethanol precipitated and purified to nucleotide resolution on denaturing 12% polyacrylamide/8 M urea gels. The full length transcripts were electroeluted from the gel using a Biotrap electroelution

apparatus (Schleicher and Schuell, Dassel, Germany), ethanol precipitated, washed and dissolved in water.

### Cloning and *in vitro* transcription of synthetic yeast tRNA<sup>Asp</sup> genes

Plasmids containing synthetic genes of wild-type and mutant tRNA<sup>Asp</sup> downstream of a T7 RNA polymerase DNA dependent transcriptional promoter, were prepared as described elsewhere (24). They contain a *Bst*NI restriction site at the precise end of the sequence encoding the tRNA. In order to permit transcription of these synthetic genes, base-pair U<sub>1</sub>·A<sub>72</sub> was replaced by G<sub>1</sub>·C<sub>72</sub> (24). Transcripts were synthesized by incubation of *Bst*NI-linearised plasmid (15 µg) with 450 units of T7 RNA polymerase in the same reaction mixture as for tRNA<sup>His</sup>.

### Cloning and *in vitro* transcription of minihelices

Plasmid pTYAA encoding the amino acid accepting arm of the tRNA-like structure present at the 3' end of the Turnip Yellow Mosaic Virus RNA, adjacent to the T7 RNA polymerase promoter was created by oligonucleotide-directed mutagenesis of clone pTYAlu as described earlier (18). *In vitro* transcription of this plasmid leads to TYA<sub>4</sub>·U<sub>22</sub>. Variants of this molecule were obtained by *in vitro* transcription of synthetic genes constructed with overlapping oligonucleotides and cloned according to established methods (24). Transcription was performed in the same conditions as for yeast tRNA<sup>Asp</sup>. Full-length transcripts were purified on 16% preparative polyacrylamide gels and electroeluted as described for tRNA<sup>His</sup> transcripts.

### Aminoacylation assays and interpretation of kinetic data

Aminoacylation assays were performed at 30°C in 55 mM Tris-HCl pH 7.5, 15 mM MgCl<sub>2</sub>, 10 mM ATP, 2.5 mM glutathione, 30 mM KCl, 25 µM L-[<sup>14</sup>C] histidine and the required concentrations of transcript and yeast HisRS. Aliquots were spotted on 3MM Whatman paper at different times and 5% trichloroacetic acid precipitated. Incorporation of radioactive aminoacid was measured by liquid scintillation spectroscopy. Kinetic parameters ( $K_M$  and  $V_{max}$ ) were determined from Lineweaver-Burk plots.

The efficiency of aminoacylation of variants respective to the transcript considered as control was appreciated by comparison of the ratios ( $V_{max}/K_M$ ). Thus,  $(V_{max}/K_M)_{relative} = (V_{max}/K_M)_{variant}/(V_{max}/K_M)_{control}$ . A more intuitive number,  $L$ , indicates the loss in aminoacylation efficiency of a variant as compared to the control. It corresponds to the inverse of  $(V_{max}/K_M)_{relative}$  (25, 26). Conversely,  $G$ , corresponds to the gain in efficiency of a variant as compared to a control and is calculated by the ratio between  $L_{control}/L_{variant} \cdot V_{max}/K_M$  values are averages of three independent experiments; errors on  $L$  and  $G$  values are about 20%.

## RESULTS AND DISCUSSION

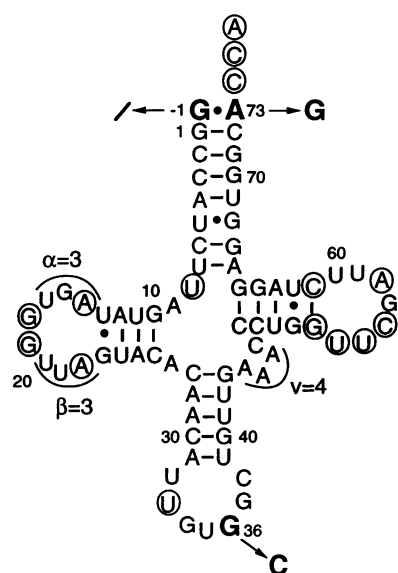
### Importance of residues G<sub>-1</sub> and A<sub>73</sub> for histidine identity in yeast

*Extra nucleotide G<sub>-1</sub>*. As occurs throughout all organisms, yeast histidine specific tRNAs differ from other cellular tRNAs by the presence of an additional residue at their 5'-end (15). Since this feature is unique and is already known to specify histidylating in *E.coli*, it represents the most obvious candidate for a major identity element in yeast tRNAs. To test this prediction, a

tRNA<sup>His</sup> transcript lacking G<sub>-1</sub> was generated (Fig. 1). As expected, the deleted variant shows its catalytic efficiency for histidylolation, estimated by the  $V_{\max}/K_M$  ratio, decreased 470-fold as compared to the wild-type tRNA<sup>His</sup> transcript (Table 1). This loss is mainly due to a decrease of  $V_{\max}$  (106-fold),  $K_M$  being only weakly affected (4-fold).

**Discriminator base 73.** Discriminator nucleotide at position 73, opposite to base G<sub>-1</sub>, is always an A in yeast tRNA<sup>His</sup> isoacceptors. Because of the frequent involvement of the discriminator position in tRNA identity and of the uniqueness of A<sub>73</sub> in yeast tRNA<sup>His</sup> isoacceptors, we considered this position as a potential histidine identity element. Since the discriminator position in eukaryotic tRNA<sup>His</sup> species is always occupied by a purine (15), a G<sub>73</sub> variant of yeast tRNA<sup>His</sup> was generated (Fig. 1). Its histidine charging ability is significantly affected with a  $V_{\max}/K_M$  value 22-times lower than for the wild-type tRNA<sup>His</sup> transcript. Again, this loss in catalytic efficiency is mainly due to a reduced  $V_{\max}$  (Table 1).

**First conclusions and their implications.** The above mutational analysis of tRNA<sup>His</sup> indicates the primordial role of residue G<sub>-1</sub>



**Figure 1.** Cloverleaf sequence of yeast tRNA<sub>1</sub><sup>His</sup> transcript. Residues that have been mutated or deleted are indicated in bold. Conserved nucleotides in all tRNAs are circled. The lengths of the a and b regions in the D-loop and the number of residues forming the variable loop are given. Unusual base pairings are symbolized by dots.

**Table 1.** Kinetic parameters for histidylolation of various transcripts derived from yeast tRNA<sup>His</sup>. The sequence of residues -1-73 is given explicitly in brackets for each transcript

Transcript	Level of charging (%)	$K_M$ ( $\mu$ M)	$V_{\max}$ (arbitrary units)	$V_{\max}/K_M$ (arbitrary units)	$L^a$ relative to transcript <sup>His</sup> (x-fold)
tRNA <sup>His</sup> (wt)	[G • A]	70	0.8	2650	3310
tRNA <sup>His</sup>	[/ • A]	57	3.6	25	7
tRNA <sup>His</sup>	[G • G]	40	1.0	150	150
tRNA <sup>His</sup> C36	[G • A]	33	0.8	1335	1670

<sup>a</sup> $L$  corresponds to losses of efficiency.

and to a lesser extent of discriminator residue A<sub>73</sub> in histidine identity in yeast. This conclusion for a eukaryotic system is reminiscent to what is found for prokaryotic tRNA<sup>His</sup> identity (11), with the exception that the discriminator residue in prokaryotes (and organelles) is always a pyrimidine instead of a purine in eukaryotes (15). Explicit proof of the role in identity of these two residues requires transplantation experiments in other tRNA frameworks. In what follows G<sub>-1</sub> and A<sub>73</sub> have been transplanted into two alternate RNA frameworks namely part of the tRNA-like domain of TYMV RNA and yeast tRNA<sup>Asp</sup>. Moreover, the differences between prokaryotes and eukaryotes was further investigated by studying the influence of pyrimidines at position 73.

### Histidylolation by yeast HisRS of minihelices derived from the TYMV tRNA-like structure

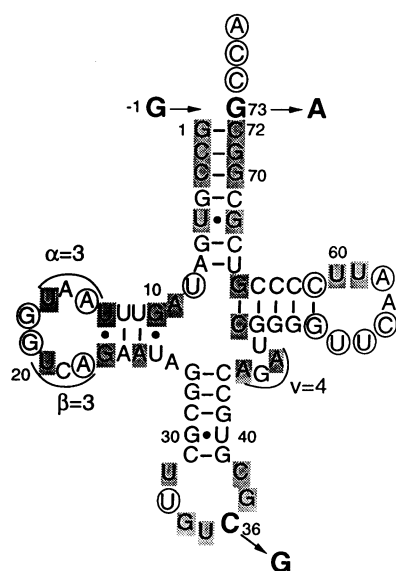
**Structural and functional background.** In our view a tRNA structure represents a scaffolding selected by nature to present identity nucleotides optimally toward the synthetase (1, 27). Thus, we have chosen to study the expression of nucleotides -1 and 73 in a RNA context markedly different from that of a canonical tRNA. This was possible because we already demonstrated that a minihelix derived from the TYMV tRNA-like structure can be charged by yeast HisRS (18). Such a minihelix contains a pseudoknot (Fig. 2). The reason accounting for its histidylolation was suggested to rely in the mimicry between nucleotide U<sub>22</sub> from the pseudoknot and residue G<sub>-1</sub> in a canonical tRNA, which makes pairing with discriminator residue A<sub>4</sub> as well as stacking of these two residues over the amino acid accepting branch (20, 28) possible. Considering the potential of this pseudoknotted structure to mimic a N<sub>-1</sub> • N<sub>73</sub> pair, we mutated the minihelix at both positions 22 (the analog of residue -1) and 4 (the analog of the discriminator base), to create in the tRNA-like context, the wild-type G<sub>22</sub> • A<sub>4</sub> mismatch of yeast tRNA<sup>His</sup>. In addition we created the three variants (G<sub>22</sub> • N<sub>4</sub>, named TY-G<sub>22</sub> • N<sub>4</sub>) with mutations at the discriminator position and the other three variants (N<sub>22</sub> • A<sub>4</sub>, named TY-N<sub>22</sub> • A<sub>4</sub>) in which the G<sub>-1</sub> mimic is changed (Fig. 2). From a practical point of view, it is noticeable that preparation of these variants with the T7 methodology is straightforward in contrast to what would occur in canonical tRNAs where the most 5'-residue has to be a G residue for efficient transcription by T7 RNA polymerase (29).

**Mutation at positions equivalent to N<sub>-1</sub> and N<sub>73</sub>.** If the G<sub>-1</sub> • A<sub>73</sub> mismatched pair is a major histidine identity element in yeast, the minihelix TY-G<sub>22</sub> • A<sub>4</sub> shown in Figure 2 should be a better substrate for HisRS than the wild-type TY-U<sub>22</sub> • A<sub>4</sub> minihelix, already known to be histidylable (18). In contrast to our earlier experiments, where conditions giving rise to optimal charging of the minihelix were used, we took in the present work the same



significantly charged by yeast HisRS (Table 3). Up to 13% of the molecules can be aminoacylated under standard aminoacylation conditions, but with a catalytic efficiency reduced 16600-fold as compared to wild-type tRNA<sup>His</sup> transcript. This decrease results predominantly from a  $V_{\max}$  effect (8800-fold). The capacity of yeast tRNA<sup>Asp</sup> transcript to be weakly histidylated is obviously relevant to the presence of one or more weak histidine identity elements (most probably present in the consensus sequence between yeast tRNA<sup>His</sup> and yeast tRNA<sup>Asp</sup>, and which concerns 42% of the nucleotides non-conserved in all tRNAs). The common conformational characteristics of both tRNAs combined to the absence of modified bases that relax tRNA structure (24, 30), contribute probably also with the capacity of yeast tRNA<sup>Asp</sup> transcript to be mischarged by yeast HisRS.

**Histidylolation of single variants.** The three yeast tRNA<sup>Asp</sup> transcripts in which histidine specific nucleotides have been inserted are displayed in Figure 3. Addition of nucleotide G<sub>-1</sub>



**Figure 3.** Cloverleaf sequence of yeast tRNA<sup>Asp</sup> transcript. Residues that were mutated or added are indicated in bold. Nucleotides common to both yeast tRNA<sup>Asp</sup> and yeast tRNA<sup>His</sup> transcripts are shaded whereas conserved residues in all tRNAs are circled. The lengths of the a and b regions in the D-loops and the number of residues forming the variable loops are given. As discussed in Materials and Methods, the tRNA<sup>Asp</sup> transcript possesses a G<sub>-1</sub>·C<sub>72</sub> instead of an U<sub>-1</sub>·A<sub>72</sub> base-pair.

at the 5'-terminus of tRNA<sup>Asp</sup> enhances markedly its histidylolation capacity. About 43% of the variant can be charged by yeast HisRS. The  $K_M$  is approximatively the same as for yeast tRNA<sup>His</sup> whereas  $V_{\max}$  is 63-fold decreased (Tables 1 and 3). Thus, this variant of tRNA<sup>Asp</sup> is 70-fold less efficiently histidylated than the tRNA<sup>His</sup> transcript, but becomes a 230-times better substrate for HisRS than the wild-type tRNA<sup>Asp</sup> transcript (Table 3). This transplantation experiment confirms the involvement of nucleotide G<sub>-1</sub> in the identity of yeast tRNA<sup>His</sup>.

Transplantation of histidine identity element A<sub>73</sub> into yeast tRNA<sup>Asp</sup> enhances also its histidylolation properties. This transcript is charged to a plateau level of 37% whereas the wild-type G<sub>73</sub> tRNA<sup>Asp</sup> molecule is only charged to 13% (Table 3). Here also, the 5-fold improvement of histidylolation efficiency is due to a  $V_{\max}$  increase. This effect is moderate and points to a minor role played by the discriminator nucleotide A<sub>73</sub> in histidine identity.

The anticodon region in histidine specific tRNAs should not *a priori* contain strong histidine identity elements because of the predominant role of the amino acid acceptor stem in this identity, well accounted for by the charging of histidine minimalist RNA substrates (this work and *e.g.* refs 13, 14, 18). However, anticodon loops of yeast tRNA<sup>Asp</sup> and tRNA<sup>His</sup> share exactly the same sequence except at position 36 which is occupied by a guanine in tRNA<sup>His</sup> and by a cytosine in tRNA<sup>Asp</sup> (see Figs. 1 and 3). Thus, introduction of G<sub>36</sub> in tRNA<sup>Asp</sup> gives rise to a chimeric tRNA molecule identical to tRNA<sup>His</sup> at the anticodon loop level. Histidylolation of this mutant is only 2-times more efficient than wild-type tRNA<sup>Asp</sup> (Table 3), showing a very low contribution of position 36 in yeast histidine identity. To directly prove this conclusion, the complementary experiment was performed in which a yeast tRNA<sup>His</sup> transcript bearing a C<sub>36</sub> in the anticodon was tested in histidylolation. As could be expected, the  $V_{\max}/K_M$  of this mutant was only 2-fold decreased as compared to the wild-type tRNA<sup>His</sup> (Table 1).

**Histidylolation of tRNA<sup>Asp</sup> double mutant G<sub>-1</sub>·A<sub>73</sub>.** The previous results have shown that both nucleotides G<sub>-1</sub> and A<sub>73</sub> contribute to histidine identity although with different strengths. In order to cumulate the beneficial effect brought by these two residues and to obtain a more efficient substrate for yeast HisRS, a double mutant derived from the tRNA<sup>Asp</sup> sequence was created presenting simultaneously G<sub>-1</sub> and A<sub>73</sub> at the top of the amino acid acceptor stem. In the case of an additional behaviour of the two identity elements, a 1000-fold gain (*G*) in histidylolation efficiency of the transplanted tRNA<sup>Asp</sup> is expected [*G* = 230×5, indeed, a *G* of 230-fold is expected to occur after

**Table 3.** Kinetic parameters for histidylolation of transplanted yeast tRNA<sup>Asp</sup>. The sequence of residues -1·73 is given explicitly in brackets for each molecule

Transcript	Level of charging (%)	$K_M$ ( $\mu M$ )	$V_{\max}$ ( $\times 10^3$ ) (a.u.)	$V_{\max}/K_M$ ( $\times 10^3$ ) (a.u.)	$L^a$ relative to transcript <sup>His</sup> (x-fold)	$G^b$ relative to transcript <sup>Asp</sup> (x-fold)
tRNA <sup>Asp</sup> (wt)	[/-G]	13	1.5	300	200	16600
tRNA <sup>Asp</sup>	[G·G]	43	0.9	42000	46700	70
tRNA <sup>Asp</sup>	[/-A]	37	2.4	2300	960	3450
tRNA <sup>Asp</sup> G36	[/-G]	30	2.7	1200	445	7440
tRNA <sup>Asp</sup>	[G·A]	35	4.0	25600	6400	520

<sup>a</sup> $L$  corresponds to losses of efficiency and <sup>b</sup> $G$  corresponds to gains of efficiency.  
a.u.: arbitrary units.

insertion of nucleotide G<sub>-1</sub> and a G of 5-fold after insertion of residue A<sub>73</sub>; in the case of additive effects, the total gain of efficiency is the product of both individual gains, in that of cooperative or anti-cooperative effects of both mutations, the total gain in efficiency is different (25, 26). Surprisingly, the double mutant is only 30-times better histidylated than yeast tRNA<sup>Asp</sup> transcript. This reflects a strong anti-cooperative behaviour of the G<sub>-1</sub> and A<sub>73</sub> histidine identity elements in the tRNA<sup>Asp</sup> context. This anti-cooperative effect is also illustrated when comparing the strong loss of histidylation efficiency of the tRNA<sup>Asp</sup> double mutant to tRNA<sup>His</sup> (*L* = 520). A likely interpretation for the anti-cooperative properties of this tRNA<sup>Asp</sup> double mutant may be a non-optimal presentation of the G<sub>-1</sub>·A<sub>73</sub> histidine identity elements towards yeast HisRS in the tRNA<sup>Asp</sup> context. This would lead to an incorrect orientation of the CCA terminus, reflected by the major contribution of V<sub>max</sub> in the loss of specificity. At the present stage of our investigations, we cannot distinguish between the precise possible structural reasons of this effect. It could be due to faint differences in the conformation of the amino acid acceptor stems of tRNA<sup>Asp</sup> and tRNA<sup>His</sup> (notice however the high homology of these regions in both tRNAs), but more likely could arise from more global changes linked to differences in the tertiary interaction networks in both tRNAs. An explicit example where the nature of nucleotides in a tertiary interaction is important for identity has been demonstrated in the case of *E.coli* tRNA<sup>Cys</sup> with the involvement of the unusual G<sub>15</sub>·G<sub>48</sub> Levitt base-pair (10). Further tRNA engineering experiments will unravel this problem in the histidine system.

## GENERAL CONCLUSIONS

This work illustrates the limitation of the transplantation approaches, in which the reality of identity elements is demonstrated by their effective expression in another tRNA sequence. Although this approach proved to be extremely useful in many instances (e.g. 31–34), it did not always allow optimal expression of an identity set in a new tRNA context. This was shown in several cases (e.g. 7–9) and is further illustrated here with the transplantation of histidine identity elements into yeast tRNA<sup>Asp</sup>. Poor activities, corresponding phenomenologically to anti-cooperative effects, are due either to antideterminants effects and/or to subtle conformational differences between the various tRNAs. Nature retained optimal structural scaffoldings during evolution to ensure optimal specificity of tRNA aminoacylation reactions.

This paper shows that the identity of tRNA<sup>His</sup> in yeast is ensured essentially by a similar molecular strategy as in prokaryotes studied so far (*E.coli*), namely a role for residues –1 and 73. The major histidine identity element in yeast is residue G<sub>-1</sub>, whose effect is correlated with that of the discriminator residue 73. Anticodon-loop nucleotides are likely not involved in histidine identity. Interestingly, yeast HisRS recognizes residues G<sub>-1</sub> and A<sub>73</sub> in particular substrates. We have shown previously that a minihelix corresponding to the aminoacyl acceptor arm of the tRNA-like domain of TYMV is efficiently charged with histidine (18). Here we demonstrate that this aminoacylation specificity is directly linked to the presence of residues mimicking nucleotides G<sub>-1</sub> and A<sub>73</sub> from yeast tRNA<sup>His</sup>, confirming the role of these nucleotides in histidine identity. Thus, HisRS recognizes its specific identity elements whether they are presented in a classical RNA helix or in a

particular folding of an RNA chain as is the case in the TYMV derived minihelix where a pseudoknot occurs.

Because histidine identity in prokaryotes is dependent upon the presence of residue C<sub>73</sub> whereas in eukaryotes it is dependent upon the presence of A<sub>73</sub>, it can be suggested that the discrimination between histidine specificity in both kingdoms is linked to the nature of the discriminator base. As far as minor histidine elements are concerned, it is interesting to notice that all the RNA substrates studied in this work contain the same base-pairs at positions 2·71 and 3·70 as in *E.coli* tRNA where these pairs have been found as minor identity elements (14).

It is worth mentioning that yeast HisRS histidylates also the tRNA-like structure of tobacco mosaic virus (16, 21), that of the satellite of this virus (19), as well as that of brome mosaic virus and a series of derived mini-substrates (20). The common feature of these substrates and of those studied in this work is the presence of a nucleotide mimicking a –1 residue. The nature of this –1 residue is variable since all four nucleotides allow histidylation although at variable efficiencies. The outcome of these observations is that histidine identity primarily requires a structural information in its substrate, the presence of a residue at position –1. Because this residue is mimicked by an internal nucleotide in the pseudoknot containing substrate, it can be concluded that this residue has to be stacked on top of the accepting helix and likely that the tRNA does not undergo drastic conformational changes at this level during aminoacylation. The fact that HisRS belongs to class II synthetases (35) agrees with this view. Indeed, in the representative class II tRNA<sup>Asp</sup>/AspRS complex structure (36), the tRNA maintains its initial conformation in the aminoacyl accepting stem in contrast to what happens in a class I complex (37). Optimal expression of the histidine identity is ensured by the adequate sequence combination between residues –1 and 73. Following this view, it is understandable that other combinations than those found in the natural tRNA<sup>His</sup> substrate of yeast HisRS are found in the viral tRNA-like molecules which have not been optimized for a yeast system.

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